Research Facilities of the RADIOBIOLOGICAL LABORATORY BUREAU OF COMMERCIAL FISHERIES Beaufort, North Carolina

UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Circular 298

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RESEARCH FACILITIES OF THE RADIOBIOLOGICAL LABORATORY BUREAU OF COMMERCIAL FISHERIES

Beaufort, North Carolina

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ABSTRACT

The history, facilities, and organization are discussed. Research is performed on estuarine ecology, biogeochemistry, pollution, and effects of radiation.

INTRODUCTION

The Radiobiological Laboratory is concerned with research that can be summed up in the term "radioecology." Radioecology is a relatively new term that refers to the study of radioactivity in our environment and the use of radioactive elements in ecological studies. Although the term radioecology was coined only recently, radioactivity has been present in man's environment since the earth was formed, and references to principles that are ecological in nature appeared in writings of the early philosophers. The need for this "new" division of ecology, however, was brought about by technological advances of modern man. Radioactivity has been added to the environment by the explosion of nuclear weapons in the atmosphere and by the release of radioactive wastes into streams, estuaries, and the oceans. Manmade radioactivity did not begin to appear in estuaries and the oceans until 1945.

The Bureau of Commercial Fisheries is vitally concerned with any situation that might adversely affect our fishery resources. The introduction of radioactive materials into the aquatic environment might constitute such a situation, and those in the Bureau who so quickly saw the need of a Radiobiological Program to study these problems deserve much credit for their insight. Although interest in radiobiology is now widespread and many organizations in this country and throughout the world are engaged in radiobiological research, the Bureau's program carried on at the Radiobiological Laboratory was one of the first and continues to be unique in many respects.

History

The present aims are an expansion of the objectives outlined in September 1948. The following statements were included in the project outline submitted to the Atomic Energy Commission (AEC) in November 1948.

"Objective:

(a) To ascertain the levels of activity which will accumulate in various invertebrate animals of littoral marine waters by the selective absorption of radioactive ions.
(b) To investigate the avenues of access to marine organisms of radioactive materials through direct absorption or ingestion of dissolved or suspended fission products, their transfer through different levels in the food chain and their ultimate removal through metabolism, decay, sedimentation, dilution, or transport."

This proposal was submitted under the title "A survey of accumulation of radioactive materials by marine invertebrate animals." A supplemental program entitled "Uptake of radioactive elements by fish, particularly marine fish" was established in 1954, and another project entitled "The effects of ionizing radiation on marine fishery organisms" was added in 1956.

Most of the earlier work was concerned with the filter-feeding lamellibranch mollusks--oysters, claims, and scallops. Emphasis was therefore placed on experimental work with marine phytoplankton--the food of these mollusks. Also, some studies were carried on during the first 2 years on the accumulation and retention of separated fission products by the commercially important blue crab. Small tanks and aquaria were used for these experiments, and possible food-chain patterns were interpreted from data on accumulation in tests based on single species or limited segments of a food chain.

When the program began in 1948, interest was great in the effects of contamination of coastal waters, harbors, and estuaries from atomic bombs, such as were being tested in

Figure 1.--Research activities and laboratory facilities from 1949 to 1964.

Figure 2.--Oysters, which can be seen through the clear sides of a modified "Lund" trough, are held in individual compartments and can be fed selected diets of radioactive foods. In such a system, an oyster's preference for certain species of phytoplankton can be accurately determined, along with its ability to digest and utilize them.

the Pacific. Radiobiologists believed that fission products would be of chief concern. Because of this belief, the experimental work was concerned first with individual fission products, and later with fission-product mixtures. Because scintillation counting techniques had not then been developed, identification of beta-emitting radionuclides accumulated by marine organisms from fission-product mixtures was laboriously determined by the use of aluminum-absorption data.

With the discovery of the importance of the accumulation of zinc 65 in tuna and cobalt 60 in mollusks collected from the Pacific, emphasis was shifted somewhat to include accumulation and retention of neutron-induced radionuclides by invertebrate species under study. Along with this research there was a shift in counting techniques to make use of the more efficient gamma detectors (scintillation) being developed. Because of the great interest in contamination of tuna that was being reported, a supplemental project requested by the AEC was initiated to emphasize accumulation by fishes. The large fish-holding tanks at the laboratory were rebuilt, the laboratory boat was fitted for collecting and transporting fish, and a boat crew was employed.

Figure 3.--Radioisotopes can be accumulated by marine animals from their food. In this study, zinc 65 and chromium 51 are being followed through four trophic levels of a marine food chain. Newly hatched brine shrimp (second trophic level) are being removed here from hatching trays and placed in flasks where they feed on radioactive phytoplankton (first trophic level). After 24 hours, the brine shrimp are fed to postlarval fish (third trophic level), which in turn are fed to mummichog (fourth trophic level).

Figure 4.--Research on living organisms has required the development of methods of catching and transporting live animals to the laboratory. The boat shown here was rigged and trolling for tuna. Fish weighing as much as 15 to 20 pounds were caught, brought to the laboratory in good condition, and held in laboratory tanks for as long as 5 to 6 weeks while the accumulation of radioactivity in the fish was being followed.

After we had obtained considerable information on both the accumulation and retention of fission products and several other radionuclides by the adult stage of commercially important fish and shellfish, we thought it important to include studies on these species at other stages of development and under environments that might represent certain periods during their life histories. Since it is important to know what damage occurs to organisms from accumulated radionuclides, especially in the early stages of development of the fishery organisms that are the most sensitive to radiation, a new project was begun. Funds supplied by the Saltonstall-Kennedy Act were used to support research to measure the damaging effects of radiation on commercially important fish and shellfish and their food organisms. The laboratory was enlarged to provide salt-water culture rooms for growing larvae, a controlled-temperature room, a histological preparation room, and office space for an increased staff.

In the research proposal for fiscal year 1962, we emphasized that a research facility should bring together investigators with sufficient diversity in education and training to take a broader approach to the solution of biological aspects of radioactive pollution in the marine environment than had been undertaken by any one organization up to that time. We further suggested that our organization attempt the team approach that is obviously necessary to accomplish the above goal. In addition to advocating a team appraoch, we also expanded our laboratory research to include pilot-type experiments in tanks and ponds and ecological studies in estuaries.

Investigations along these lines of research have continued to the present. We have stressed

Figure 5.--The effect of environmental factors, such as temperature and salinity, on the accumulation of radioactivity by invertebrates is studied under controlled conditions in the laboratory. Investigators remove organisms from the experimental environment, analyze the live organisms for radioactivity content, then return them to the aquaria. The effect of temperature and salinity on the accumulation of the radioactivity by the organisms is evaluated by statistical analysis of the data.

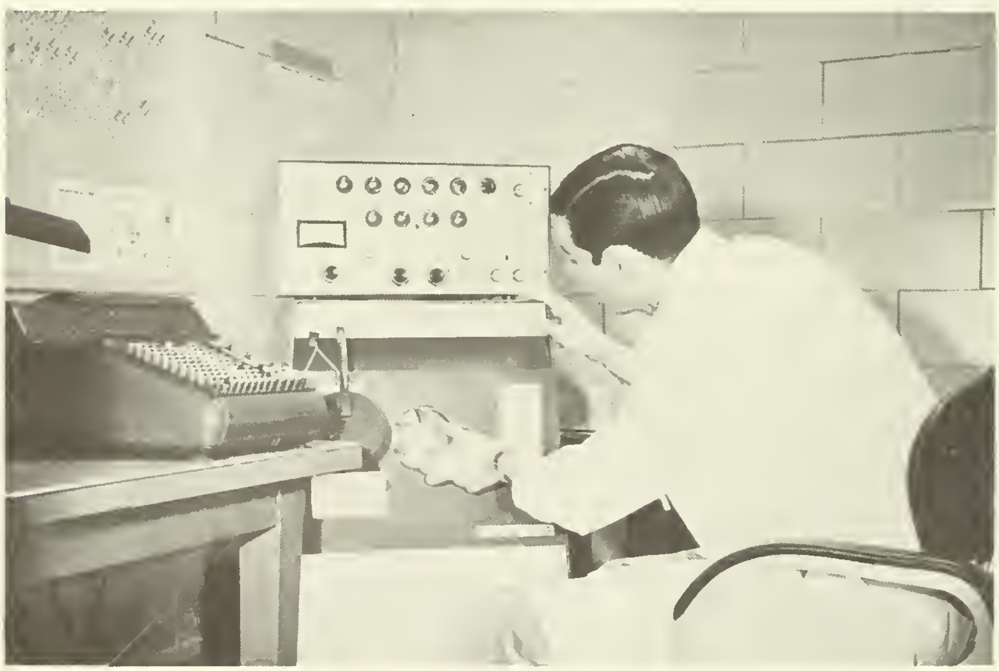


Figure 6.--Radioactivity content of organisms used in laboratory experiments is measured with specially designed electronic equipment containing a scintillation detector. Here the investigator is placing a plastic container holding one oyster into a chamber where the amount of radioactive zinc accumulated by the oyster can be measured.

Figure 7.--The rate at which carbon is converted into organic matter by marine phytoplankton--primary production--can be measured by use of carbon 14. Investigator is preparing to analyze phytoplankton cells for their carbon 14 content with a gas-flow proportional detector equipped with an automatic sample changer.

the need for collecting data under experimental conditions that would enable us to make predictions of dangers that might arise from intentional or accidental pollution of an estuary or ocean area. Emphasis was given to (1) flow systems which make possible the duplication of a natural environment in growing phytoplankton in the laboratory, (2) the simultaneous accumulation of radionuclides by sediments and the biota held in relatively large volumes of sea water, (3) total-element measurement so that specific activity in the different components of a community can be compared, (4) total-animal counting so that long-term community studies are not disrupted by sacrificing the animals for radioactivity measurements, (5) observations of factors influencing the cycling of radionuclides in estuaries, and (6) the effects of external and internal radiation. Also, it was hoped that a better understanding of the cycling of many radionuclides in the estuary would be obtained by placing increased effort on studies of energy flow. The rate at which energy flows through the biological food chain no doubt influences the rate at which many radionuclides circulate from environment to organism and back again.

Future research will attempt to understand the factors that control production of fishery organisms in estuaries. Natural estuarine ecosystems, however, are complex, and a study of the dynamic aspects of a complete ecosystem is difficult. Thus, need will exist to develop a mathematical model that can be used to assess the behavior of the system. The development of complex models simulating the relations among animals, plants, their uptake of materials, and the flow of energy from the estuarine environment requires the use of computers. The capacity of computers to integrate large quantities of diverse data will make it possible to evaluate new data continually as they are collected. Also, it will be possible to resolve many problems that have proved, thus far, to be too difficult to solve by methods used in the past.

Facilities

The present laboratory facilities, occupied in July 1964, consist of three buildings. One building is a two-story laboratory of about 20,000 square feet, the second is a radiation laboratory of 1,500 square feet, and the third, of 1,000 square feet, provides storage and contains a crematory for ashing radioactive organisms. The main building has office and laboratory space for about 16 investigators and supporting staff, two large salt-water laboratories, three constant-temperature rooms, several counting and instrument rooms, a stockroom, a conference room, and offices for administrative staff. The radiation building is divided into three parts: a radiation room with 3-ft. concrete walls and running salt water for studies of chronic effects of low-level irradiation; an instrument room for the cobalt 60 irradiator, X-ray machine, and neutron generator; and an aquarium room with running salt water for maintaining experimental animals.

Figure 8.--View from secretary's desk looking down hall of first floor. Portion of lobby is to the right of the hall.

Figure 9.--Salt-water facilities, showing large fiberglass storage tanks (upper right-hand corner) and three sizes of fiberglass tanks inside of laboratory. As far as we can determine, this is the first use of large fiberglass tanks as storage tanks for sea water. Lower right-hand picture shows fiberglass-covered wooden table. Salt-water system consists of a dual set of PVC (polyvinyl chloride) pipes from storage tanks to inside of laboratory. The dual pipe system is alternated weekly--one system is used for salt water while the other system is held as a standby with fresh water for killing organisms that foul the pipes.

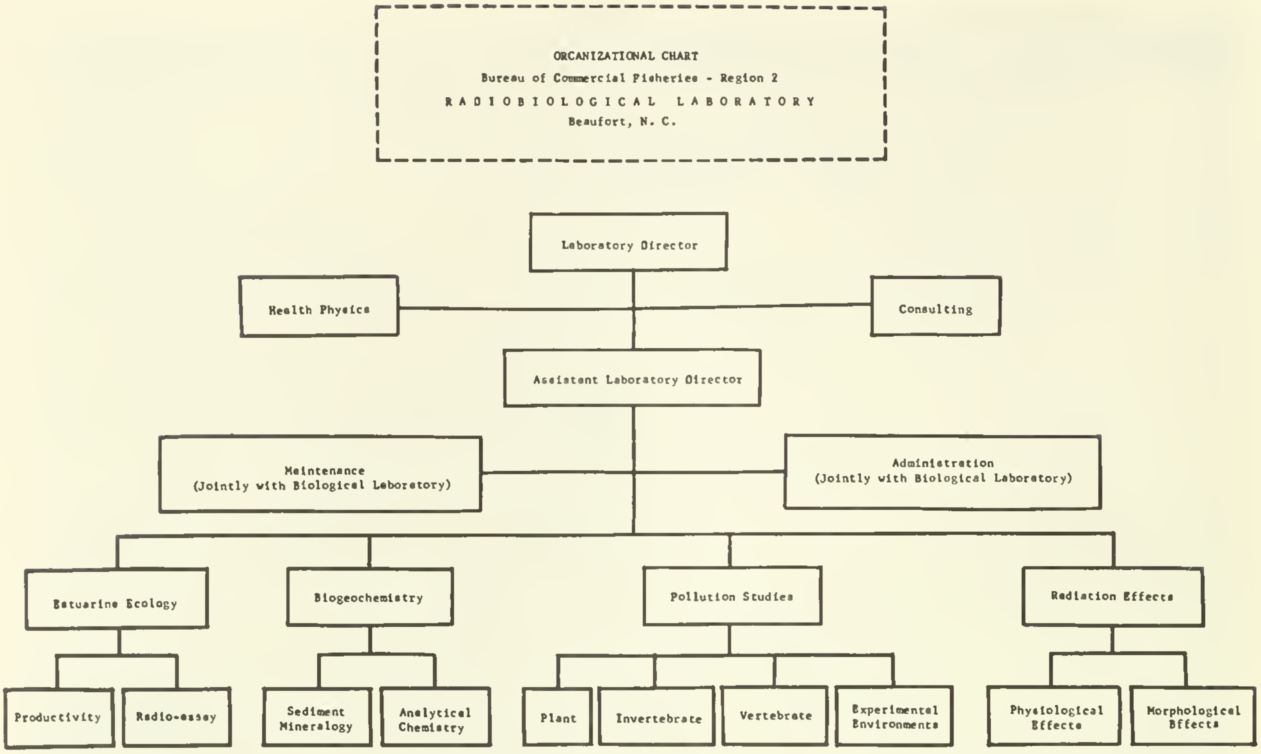


Figure 10.--Organizational chart.

Organization

The widespread use of isotopes produces a host of related problems that can be solved more easily by investigators in related fields working in the same laboratory than can be accomplished by individual investigators working at separate locations. A group of investigators of varied background can offer (1) a team approach in solving problems, (2) more efficient use of costly equipment needed for this work, (3) continuity to the research work, (4) convenience for sponsors of research, and (5) a much broader approach in the research than is available to an individual investigator. The staff of the Radiobiological Laboratory has been organized with these points in mind.

The research of the Radiobiological Laboratory is divided into the broad areas of estuarine ecology, biogeochemistry, pollution studies, and radiation effects (fig. 10). These four areas of research are called programs. Within each program are a number of projects. The considerable freedom given each project leader in planning the details of his research results in a wide variety of studies. For example, there are projects concerned with productivity of estuaries, trace elements in sea water, and mathematical modeling. The four program chiefs, along with the laboratory director, consider the broader aspects of the research program and decide when two or more programs should cooperate in solving problems too general to be handled by a single program project.

RESEARCH ACTIVITIES

The purpose of research at this laboratory is twofold: (1) to determine the fate (cycling) of radioactive elements that are released into the estuarine environment and the effect of this radioactivity on estuarine plants and animals; and (2) to develop and apply radioisotopic methods to studies of estuarine ecology. We are investigating basic problems in ecology, radiobiology, biochemistry, and geochemistry to obtain a more comprehensive understanding of the accumulation of radionuclides by fishery organisms. Because ionizing radiations from radionuclides interact with other environmental factors to affect the growth and survival of fishery organisms, we determine the responses of the biota to radiation under various environments. Also, radioisotopes are used to trace the movement of elements in the estuarine environment. This movement is a cyclic exchange between biotic and abiotic phases in the environment. With radioisotopes we can determine the rates at which this exchange takes place and also determine the amounts of elements concentrated by organisms as the elements are passed through the food web.

MEASURING PRIMARY PRODUCTIVITY IN ESTUARIES

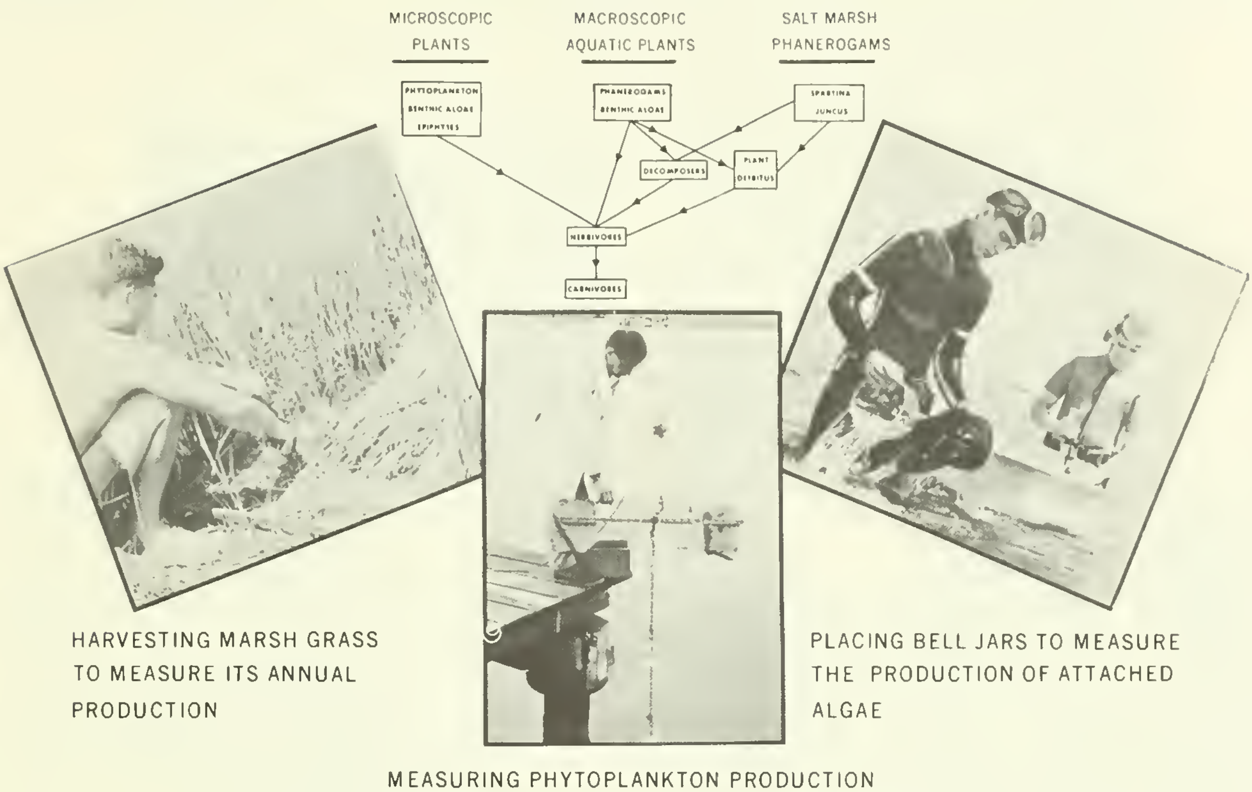


Figure 11.--The amount of energy flow through an ecosystem depends on the sunlight energy fixed by photosynthesis in producer organisms; all other organisms ultimately depend on this source for food. In the estuarine environment, primary producers can be separated into three broad categories--microscopic floating plants (phytoplankton), rooted plants (marsh grass), and macroscopic "attached" plants (benthic algae). Investigators are measuring the amount of energy (carbon) fixed by these primary producers by: measuring the uptake of radioactive carbon by phytoplankton; harvesting marsh grass and measuring its annual growth; and measuring rates of photosynthesis of algae attached to bottom sediments by isolating sediment, algae, and water with a bell jar. Changes in dissolved oxygen in the water enclosed by the bell indicate rates of photosynthesis.

Research at the laboratory is separated into four programs--three concerned with cycling of nutrient elements and their radionuclides, and one with the effects of radiation on marine organisms.

Estuarine Ecology

The Estuarine Ecology Program investigates the biological productivity of estuaries. The ultimate aim of this research is prediction of the fate of radionuclides introduced into the estuarine environment--especially their accumulation by organisms consumed by humans. Accuracy in estimating this accumulation in edible species requires, however, knowledge of the pathways and mechanisms of accumulation for the entire ecosystem. Shallow estuaries are different from--and in some ways more complex than--the open sea, because of the ease with which materials may move between the water and the sediment, and the presence of food chains based on primary producers other than phytoplankton. Work is being done on the rate of primary production by phytoplankton, attached algae, and higher plants, and on transfer of this production to other trophic levels, because the flow of energy influences the cycle of materials and thus the movement of radionuclides.

Biogeochemistry

The rapid accumulation of certain radionuclides by estuarine organisms reflects the metabolism of trace elements. Complete understanding of the cycling of radionuclides in the estuary requires knowledge of the elemental composition of estuarine organisms; the transport processes operating in the organism to incorporate the elements; and the physiological disposition, i.e., the metabolism, of the elements.

Monitoring Fallout Radioactivity in Estuarine Organisms

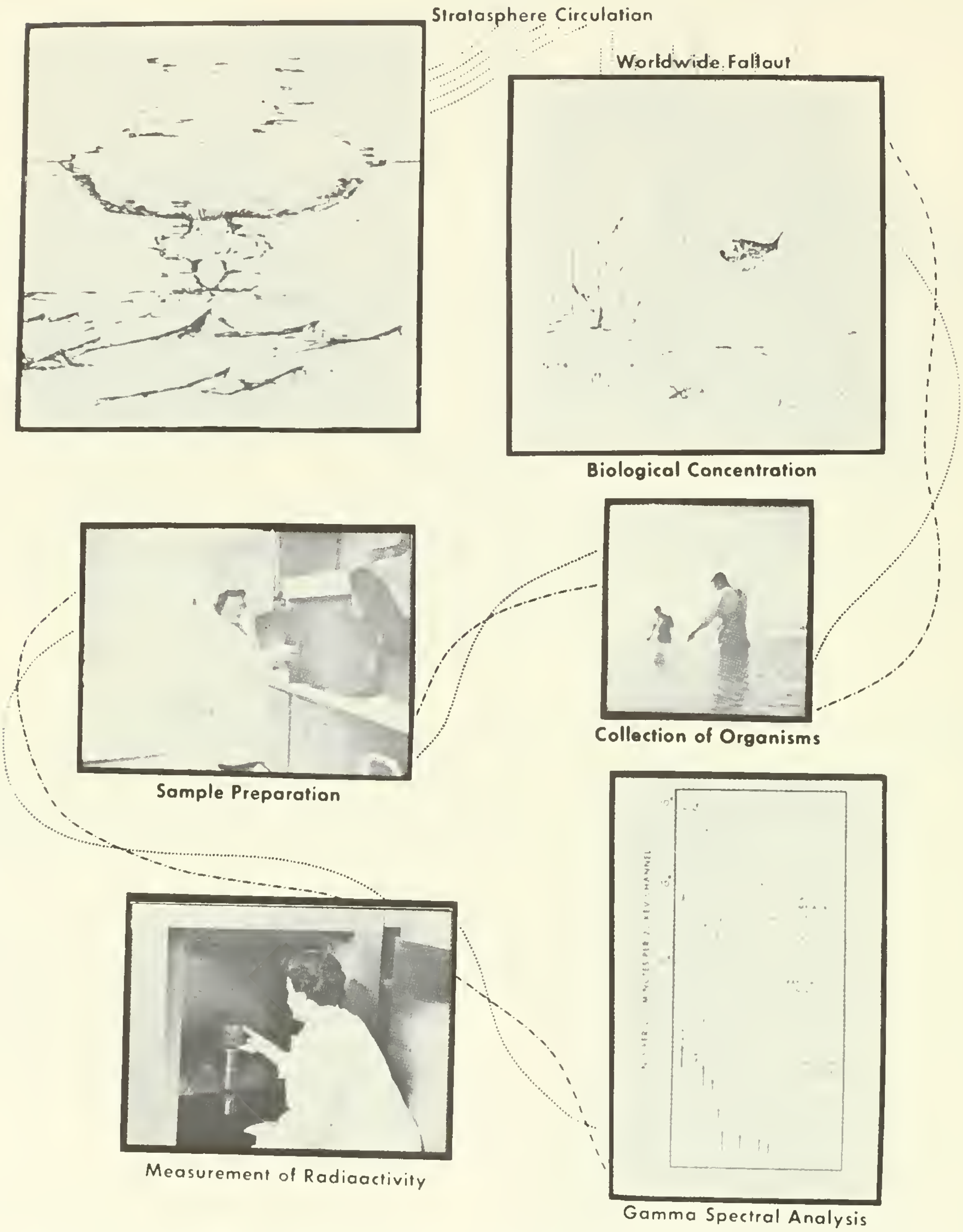


Figure 12.--Radioactive materials are added to the estuarine environment through fallout from the explosion of nuclear weapons. These materials often are accumulated and concentrated by seafood organisms. As part of a program to study the biological concentration of fallout radioactivity by estuarine organisms, investigators collect organisms in the natural environment and take them to the laboratory for analysis. The amount and type of radioactivity in the organisms are measured with specially designed electronic equipment. The radioactivity content of some fresh-water and estuarine clams is shown in gamma spectral analyses.

USING RADIOISOTOPES IN FISHERY RESEARCH

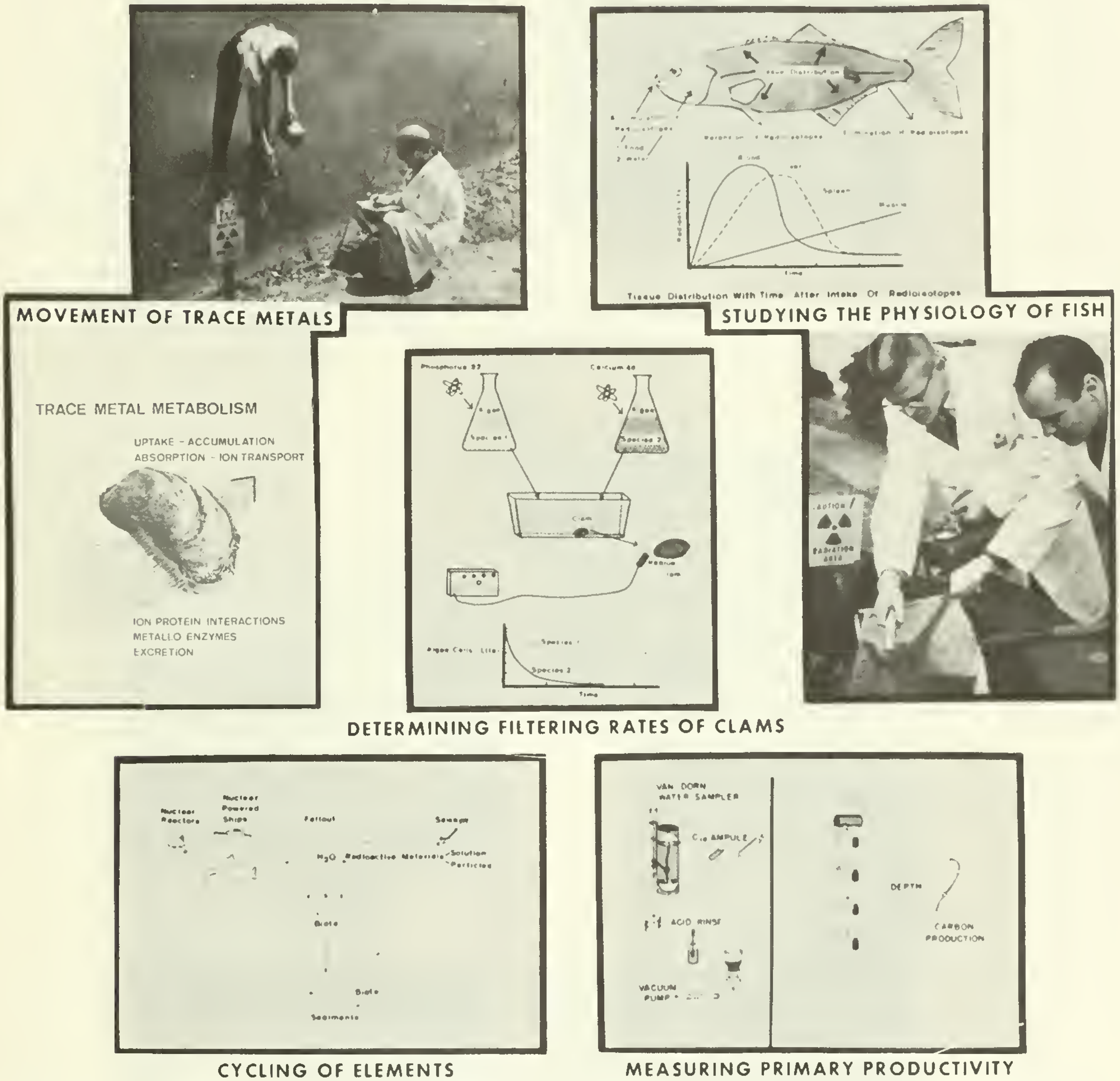


Figure 13.--New research possibilities have arisen in the field of ecology as radioactive tracers have become available. The movement of trace elements through experimental ponds is followed by using radioactive isotopes of the elements as tags or labels. The capacity of clams to filter food from water is measured through the use of algae labeled with radioactive elements. Also, the rate at which carbon is produced by plants--primary production--can be determined by rate of uptake of carbon 14 by the plants.

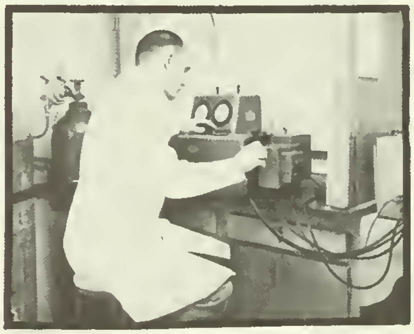
STUDYING EFFECTS OF RADIATION

USING COBALT 60 IRRADIATOR

INSERTING FISH INTO IRRADIATOR

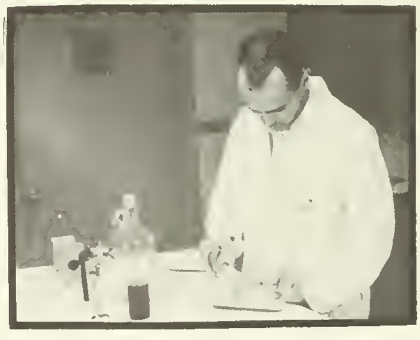
IRRADIATING MARINE ORGANISMS WITH COBALT 60

BIOCHEMICAL DETERMINATIONS



MEASURING TOTAL ELEMENT

MEASURING RESPIRATION



DETERMINING PROTEINS

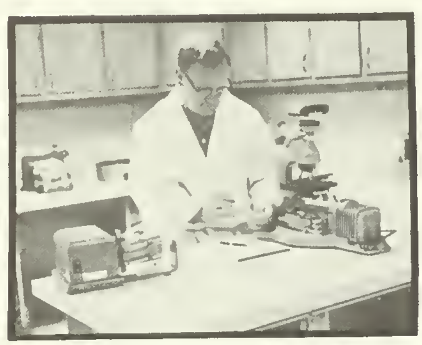
ON ESTUARINE ORGANISMS

REMOVING BLOOD

CELLULAR CHARACTERISTICS

COUNTING CELLS

MEASURING PACKED CELL VOLUMES



DETERMINING CELL TYPES

Figure 14.--The effect of ionizing radiation on estuarine organisms is studied at the Radiobiological Laboratory. Organisms are exposed to gamma radiation from a cobalt 60 source which is contained in a specially built irradiator. After exposure to radiation, the organisms are subjected to various biochemical and cellular tests. Often, radiation-induced physiological changes occur first in the blood.

We are now studying the trace element composition of estuarine organisms, with particular reference to those organisms known to concentrate environmental radioactivity. Mineral metabolism in the American oyster is being studied with special emphasis on protein-metal interactions. Anatomical and subcellular distribution and the physiological function of various minerals are being analyzed. Soluble metalloproteins are chromatographed, and the protein fractions are characterized by ultraviolet absorption, nitrogen content, mineral content, and enzymatic activity.

Estuarine organisms and sediments must also be characterized chemically before conclusions on accumulation or exchange of radioisotopes can be drawn. Estuarine organisms are being systematically collected and analyzed for naturally occurring gamma activity; biological indicators of radioactivity are identified and studied to determine the physiological characteristics which cause them to accumulate specific radionuclides. Environmental samples collected for radioassay are analyzed for content of various trace metals by atomic absorption spectrophotometry. This project is being expanded to include facilities for additional elements so that our knowledge of the cycling of elements and their radionuclides in the estuarine environment can be correlated with compositional data.

Pollution Studies

Experiments are conducted in this program to explore the routes and rates by which radioactivity released into the estuarine environment might be returned to man and to determine the rates by which nutrients move through the biotic and abiotic phases in the environment. Scope of research ranges from experiments in the natural environment with communities of organisms to those conducted in the laboratory with single species of organisms maintained in small volumes of water.

Observations are made on the cycling of radionuclides released in artificial tidal ponds and other more natural environments. By following the uptake of the radioisotopes by the biota, we can compare the rates of accumulation by the different species of organisms because they are all exposed to the same environmental factors. The rates should be approximately those which would occur in the sea because the physiological condition of the organisms should be similar to their normal conditions. Samples of the biota, sediment, and water are removed from the pond periodically and analyzed for radioactivity. Organisms are analyzed alive and returned to the pond so that the ecology of the pond will be unchanged. At the end of the experiment, components of the pond are analyzed for content of stable elements so that their specific activity can be calculated.

All processes in an estuary cannot be studied in situ; therefore, experiments also are conducted under controlled conditions in the laboratory. For example, the interaction of environmental factors such as pH, temperature, salinity, and stable element concentration on the exchange of radionuclides and nutrients between sediments and sea water and the accumulation of these materials by estuarine organisms is determined by varying one of these factors while the others remain constant. Other experiments in the laboratory include studies of the retention of radionuclides by fish and shellfish and the passage of radionuclides from water to phytoplankton to organisms of other trophic levels. Analyses of related stable element content of the organisms in each trophic level are made so that the passage of a radionuclide through the food chain can be related to the specific activity of the organisms and their environment.

Radiation Effects

Research on the effects of ionizing radiation on marine organisms is a logical sequel to the investigations of the fate of radioactive materials in the marine environment. Our main objectives are to determine the influence of environmental factors on the response of estuarine organisms to ionizing radiation and to characterize the physiological effects of radiation on these organisms. We are now investigating the influences of temperature, salinity, population, number of organisms per volume, and food on the responses of estuarine organisms to radiation. In an estuary, salinity and temperature largely characterize the physicochemical properties of the water and control the fauna. Effects of temperature upon the response of mammals and fresh-water fish to radiation are well documented, but data are few concerning the effects of temperature on the response of marine organisms to radiation and, to our knowledge, no data exist on the influence of salinity.

We are describing the interactions of radiation, salinity, and temperature by subjecting various developmental stages of resident estuarine species to combinations of the three factors and measuring changes in their LD-50's (dose of radiation (in roentgens) required to kill 50 percent), growth, and respiration. Preliminary work indicates salinity can modify LD-50's of estuarine species. Newly hatched brine shrimp, exposed to different doses of radiation, are reared in various combinations of population sizes and water volumes to determine the influence of radiation on their growth and survival. Similarly designed experiments will test the influence of food supply. Results from constant low-level irradiation are to be compared with those from acute doses.

FUTURE RESEARCH

Sufficient capabilities have been developed to permit the laboratory staff, acting as a team, to mount an inclusive study of the dynamic aspects of the radioecology of a specific estuary--a case history or study in depth. Our plan of attack is first to complete a preliminary mathematical model of the flow of energy and specific materials and to begin studying the standing crop of the estuarine biota. On the basis of information obtained in the preliminary studies, an "all-inclusive" study involving as many aspects of estuarine ecology as possible will be undertaken. Information will be gathered on seasonal and developmental feeding trends, selective feeding, and competition for food among species. Data will be collected also on population dynamics, including size and composition of estuarine populations, seasonal abundances, and distribution of various species. From this study we plan to obtain information necessary to (1) increase the production of organic matter, (2) channel the available organic matter into food webs that support fishery organisms, and (3) predict the routes and rates of movement of radionuclides in the estuarine environment.

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